Amendments to the Claims

The updated claim listing below will replace all prior versions and listings of claims in the application.

(Currently Amended) A compound of formula (XII)

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₀H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

R¹⁴, R¹⁴, and R¹⁶ R¹⁴ and R¹⁵ are each independently a protecting group that is removable by an enzyme;

wherein the enzyme is an esterase or phosphatase; and

R¹¹ together with the oxygen atom to which it is attached is an ester or an acyloxymethyl ether;

with the proviso that R11, R14, and R15 are not all acetyl groups.

(Original) The compound of claim 1, wherein
 R⁷ is -CH₂-C₆H₅, naphthyl, -CH₂-C₆H₄OH, -CH₂-C₆H₄F, or -CH₂-C₆H₄OR¹⁴;

 R^8 is $-CH_2C_6H_5$, $-CH_2C_6H_{11}$, $-CH_2C_5H_9$, or $-(CH_2)_3NHC(=NH)NH_2$; and R^9 is phenyl, indolyl, $-C_6H_4OH$, $-C_6H_4NH_2$, $-C_6H_4F$, or $-C_6H_4OR^{15}$.

3. (Previously presented) The compound of claim 1, wherein -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently esters.

(Original) The compound of claim 1, wherein
R¹¹ is acetyl; and
R¹⁴ and R¹⁵ are independently butyryl, acetoxymethyl,

propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

(Original) The compound of claim 1, wherein
 R¹¹ is butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl; and

R¹⁴ and R¹⁵ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

(Currently Amended) A compound of formula (XII)

wherein R^7 is H, alkyl, heteroalkyl, aryl, or -CH₂-C $_6$ H $_4$ OR 14 ;

R⁸ is H, alkyl, heteroalkyl, or aryl;

 R^9 is H, alkyl, heteroalkyl, aryl, or $-C_8H_4OR^{15}$;

 R^{10} is -H, -CH₃, or -CH(CH₃)₂; and

 R^{44} , R^{14} , and R^{16} R^{14} and R^{15} are each independently a protecting group that is removable by an enzyme;

wherein the enzyme is an esterace or phosphatace

R¹¹ together with the oxygen atom to which it is attached is an ester or an acyloxymethyl ether; and

wherein the concentration of the compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C is reduced by less than 50% after 45 minutes.

(Original) The compound of claim 6, wherein

 R^7 is -CHz-C6H5, naphthyl, -CHz-C6H4OH, -CHz-C6H4F, or -CHz-C6H4OR $^{14};$

 R^{9} is $-CH_{2}C_{6}H_{5}$, $-CH_{2}C_{6}H_{11}$, $-CH_{2}C_{5}H_{9}$, or $-(CH_{2})_{3}NHC(=NH)NH_{2}$; and R^{9} is phenyl, indolyl, $-C_{6}H_{4}OH$, $-C_{6}H_{4}NH_{2}$, $-C_{6}H_{4}F$, or $-C_{6}H_{4}OR^{15}$.

- 8. (Previously Presented) The compound of claim 6, wherein -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently esters.
- 9. (Original) The compound of claim 6, wherein R¹¹, R¹⁴, and R¹⁶ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
 - 10. (Currently amended) A compound of formula (XII)

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R9 is H, alkyl, heteroalkyl, aryl, or -C6H4OR15;

R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are each independently a protecting group that is removable by an enzyme;

wherein the enzyme is an esterace or phosphatase

R¹¹ together with the oxygen atom to which it is attached is an ester or an acyloxymethyl ether; and

wherein the removal of at least one protecting group that is removable by the enzyme provides a parent compound; and

wherein the time necessary for the concentration of the compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced

by 50% is greater than the time necessary for the concentration of the parent compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50%.

- 11. (Currently amended) The compound of claim 10, wherein the removal of at least two protecting groups that are removable by the enzyme provides the parent compound.
- 12. (Currently amended) The compound of claim 10, wherein the removal of all protecting groups that are removable by the enzyme provides the parent compound.
- 13. (Original) The compound of claim 10, wherein $R^7 \text{ is -CH}_2\text{-}C_6\text{H}_5, \text{ naphthyl, -CH}_2\text{-}C_6\text{H}_4\text{OH, -CH}_2\text{-}C_6\text{H}_4\text{F, or -CH}_2\text{-}}$ $C_6\text{H}_4\text{OR}^{14};$ $R^8 \text{ is -CH}_2\text{C}_6\text{H}_5, \text{-CH}_2\text{C}_6\text{H}_{11}, \text{-CH}_2\text{C}_5\text{H}_9, \text{ or -(CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2; \text{ and }}$ $R^9 \text{ is phenyl, indolyl, -C}_6\text{H}_4\text{OH, -C}_6\text{H}_4\text{NH}_2, \text{-C}_6\text{H}_4\text{F, or -C}_6\text{H}_4\text{OR}^{15}.}$
- 14. (Previously presented) The compound of claim 10, wherein -OR¹¹,
 -OR¹⁴, and -OR¹⁵ are each independently esters.
- 15. (Currently amended) The compound claim 10, wherein R¹¹, R¹⁴, and R¹⁵ are <u>each</u> independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivalcyloxymethyl.
 - 16. (Currently amended) A compound of formula (XIII) or (XIV)

wherein R7 is H, alkyl, heteroalkyl, aryl, or -CH2-C6H4OR14;

R⁸ is H, alkyl, heteroalkyl, or aryl;

 \mathbb{R}^{12} and \mathbb{R}^{13} are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR 16 ; n is 0, 1, or 2; and

R¹¹, R¹⁴, and R¹⁶ R¹⁴ and R¹⁶ are each independently a protecting group that is removable by an enzyme;

wherein the enzyme is an esterase or phosphatase; and $\frac{R^{11} \text{ together with the oxygen atom to which it is attached is an ester or}{an acyloxymethyl ether.}$

17. (Original) The compound of claim 16, wherein $R^7 \text{ is -CH}_2\text{-C}_6\text{H}_5, \text{ naphthyl, -CH}_2\text{-C}_6\text{H}_4\text{OH, -CH}_2\text{-C}_6\text{H}_4\text{F, or -CH}_2\text{-}}$ $C_6\text{H}_4\text{OR}^{14}; \text{ and}$ $R^8 \text{ is -CH}_2\text{C}_6\text{H}_5, \text{-CH}_2\text{C}_6\text{H}_{11}, \text{-CH}_2\text{C}_6\text{H}_9, \text{ or -(CH}_2)}_5\text{NHC}(=\text{NH})\text{NH}_2.$

- 18. (Previously presented) The compound of claim 16, wherein -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently esters.
- 19. (Currently amended) The compound of claim 16, wherein R¹¹, R¹⁴, and R¹⁶ are <u>each</u> independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
 - 20. (Original) The compound of claim 16, wherein n is 1.
 - (Original) A composition, comprising:
 the compound of claim 1 in solution.
- 22. (Original) The composition of claim 21, wherein the solution is an aqueous solution.
- 23. (Original) The composition of claim 21, wherein the solution comprises DMSO or alcohol.
 - 24. (Original) A composition, comprising: the compound of claim 6, in solution.
- 25. (Original) The composition of claim 24, wherein the solution is an aqueous solution.
- 26. (Original) The composition of claim 24, wherein the solution comprises DMSO or alcohol.
 - (Original) A composition, comprising:
 the compound of claim 10, in solution.
- 28. (Original) The composition of claim 27, wherein the solution is an aqueous solution.
- 29. (Original) The composition of claim 27, wherein the solution comprises DMSO or alcohol.

- (Original) A composition, comprising:
 the compound of claim 16, in solution.
- 31. (Original) The composition of claim 30, wherein the solution is an aqueous solution.
- 32. (Original) The composition of claim 30, wherein the solution comprises DMSO or alcohol.
- 33. (Currently amended) A protected luminophore, which is a protected coelenterazine that includes an imidazolone oxygen protected with a protecting group that is removable by an enzyme;

wherein the protecting group together with the imidazolone oxygen to which it is attached, form an ester or an <u>acyloxymethyl</u> ether;

wherein subsequent removal of said protecting group provides a parent coelenterazine; and

wherein the time necessary for the concentration of the protected coelenterazine in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50% is greater than the time necessary for the concentration of the parent coelenterazine in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50%.

34.-38. (Canceled)

39. (Withdrawn) A method of measuring the enzymatic activity of a luminogenic protein comprising:

contacting a luminogenic protein, a deprotecting enzyme, and a protected luminophore in solution to form a composition; and detecting light produced from the composition.

40. (Withdrawn) The method of claim 39, wherein the luminogenic protein is Renilla luciferase.

41. (Withdrawn) The method of claim 39, wherein the protected luminophore is a compound of formula (XII)

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₅H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R9 is H, alkyl, heteroalkyl, aryl, or -CeHaOR15;

R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are each independently a protecting group that is removable by an enzyme.

- 42. (Withdrawn) The method of claim 41, wherein $R^7 \text{ is -CH}_2\text{-C}_6\text{H}_5, \text{ naphthyl, -CH}_2\text{-C}_6\text{H}_4\text{OH, -CH}_2\text{-C}_6\text{H}_4\text{F, or -CH}_2\text{-}} \\ \text{C}_6\text{H}_4\text{OR}^{14}; \\ \text{R}^6 \text{ is -CH}_2\text{C}_6\text{H}_5, -\text{CH}_2\text{C}_6\text{H}_{11}, -\text{CH}_2\text{C}_5\text{H}_9, \text{ or -(CH}_2)}_3\text{NHC}(=\text{NH})\text{NH}_2; \text{ and }} \\ \text{R}^9 \text{ is phenyl, indolyl, -C}_6\text{H}_4\text{OH, -C}_6\text{H}_4\text{NH}_2, -C}_6\text{H}_4\text{F, or -C}_6\text{H}_4\text{OR}^{15}.}$
- 43. (Withdrawn) The method of claim 41, wherein -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently esters.
- 44. (Withdrawn) The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
- 45. (Withdrawn) The method of claim 39, wherein the protected luminophore is a compound of formula (XIII) or (XIV)

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

 R^{12} and R^{13} are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR 16 ; n is 0, 1, or 2; and

R¹¹, R¹⁴, and R¹⁶ are each independently a protecting group that is removable by an enzyme.

46. (Withdrawn) The method of claim 45, wherein $R^7 \text{ is -CH}_2\text{-C}_6\text{H}_5, \text{ naphthyl, -CH}_2\text{-C}_6\text{H}_4\text{OH, -CH}_2\text{-C}_6\text{H}_4\text{F, or -CH}_2\text{-}}$ $C_6\text{H}_4\text{OR}^{14}; \text{ and}$ $R^8 \text{ is -CH}_2\text{C}_6\text{H}_5, \text{-CH}_2\text{C}_6\text{H}_{11}, \text{-CH}_2\text{C}_6\text{H}_9, \text{ or --(CH}_2)}_3\text{NHC}(=\text{NH})\text{NH}_2.}$

47. (Withdrawn) The method of claim 45, wherein -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently esters.

- 48. (Withdrawn) The method of claim 45, wherein R¹¹, R¹⁴, and R¹⁶ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
 - 49. (Withdrawn) The method of claim 45, wherein n is 1.
- 50. (Withdrawn) The method of claim 39, wherein the composition comprises a cell.
- 51. (Withdrawn) The method of claim 39, wherein the composition comprises a cell which contains the deprotecting enzyme.
- 52. (Withdrawn) The method of claim 51, wherein detecting light produced from the composition indicates the location of the deprotecting enzyme in a cell.
- 53. (Withdrawn) The method of claim 39, wherein the composition comprises a cell lysate.
- 54. (Withdrawn) The method of claim 39, wherein the deprotecting enzyme is an esterase.
- 55. (Withdrawn) The method of claim 39, wherein the solution is an aqueous solution.
- 56. (Withdrawn) The method of claim 39, wherein the solution comprises DMSO.
- 57. (Withdrawn) The method of claim 39, wherein the protected luminophore is a protected coelenterazine;

wherein the enol group has been converted to an ester or an ether comprising a protecting group that is removable by an enzyme.

58.-62. (Canceled)

63. (Withdrawn-Previously presented) The method of claim 62, wherein the protected luminophore is a protected coelenterazine;

wherein the enol group has been converted to an ester or an ether comprising an group that is removable by the non-luminogenic enzyme.

64. (Withdrawn) The method of claim 62, wherein the protected luminophore is a compound of formula (XII).

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁶ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

 R^{10} is -H, -CH₃, or -CH(CH₃)₂; and

 $R^{11},\,R^{14},\,$ and R^{16} are each independently a protecting group that is removable by an enzyme that are removable by the non-luminogenic enzyme.

65. (Withdrawn) The method of claim 62, wherein the protected luminophore is a compound of formula (XIII) or (XIV)

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wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R¹² and R¹³ are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR¹⁶; n is 0, 1, or 2; and

R¹¹, R¹⁴, and R¹⁶ are each independently protecting groups that are removable by the non-luminogenic enzyme.

66.-67. (Canceled)

- 68. (Currently amended) The compound of claim 1, wherein $R^{14}_{-}R^{14}_{-}$ and $R^{15}_{-}R^{14}_{-}$ and $R^{15}_{-}R^{15}_{-}$ are <u>each</u> independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.
- 69. (Currently amended) The compound of claim 1, wherein $R^{11}_{-}R^{14}_{-} \text{and } R^{16} \, \underline{R^{14}} \text{ and } \underline{R^{15}} \text{ are independently selected from the group consisting of an alkyl group containing from 1-15 carbon atoms and a heteroalkyl group containing from 1-15 carbon atoms.$
- 70. (Currently amended) The compound of claim 1, wherein R^{14} , R^{14} , and R^{16} R^{14} and R^{15} are independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein $-QR^{14}$, $-QR^{14}$, and $-QR^{16}$ $-QR^{16}$ are each independently an ester group or an ether group.
 - 71. (Currently amended) The compound of claim 10, wherein

 R^{14} , R^{14} , and R^{16} R^{14} and R^{15} are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.

- 72. (Currently amended) The compound of claim 10, wherein $R^{14}_{-}R^{14}_{-}$ and $R^{16}_{-}R^{14}_{-}$ and R^{16}_{-} are independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein $-OR^{14}_{-}-OR^{14}_{-}$ and $-OR^{16}_{-}$ $-OR^{16}_{-}$ are each independently an ester group or an ether group.
- 73. (Currently amended) The compound of claim 16, wherein $R^{14}, R^{14}, \text{and } R^{16} \, \underline{R^{14} \, and \, R^{16}} \, \text{are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.$
- 74. (Currently amended) The compound of claim 16, wherein R^{14} , R^{14} , and R^{16} R^{14} and R^{16} are independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein $-OR^{14}$, $-OR^{14}$, and $-OR^{16}$ are each independently an ester group or an ether group.
- 75. (Withdrawn) The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.
- 76. (Withdrawn) The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are independently a heteroalkyl group containing from 1-20 carbon atoms, and comprising at least one of an ester group and an ether group.
- 77. (Withdrawn) The method of claim 45, wherein R¹¹, R¹⁴, and R¹⁶ are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.
 - (Withdrawn) The method of claim 45, wherein

 R^{11} , R^{14} , and R^{16} are independently a heteroalkyl group containing from 1-20 carbon atoms, and comprising at least one of an ester group and an ether group.

- 79. (Cancelled)
- 80. (Previously presented) The compound of claim 1, wherein the protecting group is selected from the group consisting of ester, ether, phosphoryl and glucosyl.
- 81. (Previously presented) The compound of claim 5, wherein $R^7 \text{ is -CH}_2\text{-C}_6\text{H}_6, \text{ naphthyl, -CH}_2\text{-C}_6\text{H}_4\text{OH, -CH}_2\text{-C}_6\text{H}_4\text{F, or -CH}_2\text{-}}$ $C_6\text{H}_4\text{OR}^{14};$ $R^6 \text{ is -CH}_2\text{C}_6\text{H}_5, \text{-CH}_2\text{C}_6\text{H}_{11}, \text{-CH}_2\text{C}_5\text{H}_9, \text{ or -(CH}_2)}_3\text{NHC}(=\text{NH})\text{NH}_2; \text{ and }}$ $R^9 \text{ is -C}_6\text{H}_4\text{OR}^{15}.$
 - 82. (Previously presented) The compound of claim 1 of the formula:

83. (Previously presented) The compound of claim 5, of the formula:

84. (Currently amended) A compound of the formula:

where R¹¹ together with the oxygen atom to which it is attached is an ester or an acyloxymethyl ether; —and

R¹⁵ <u>is_</u>are-independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein -OR¹⁵, and -OR¹⁵ <u>is</u> are each independently an ester group or an ether group.

- 85. (Previously presented) The compound of claim 84, wherein R¹¹ and R¹⁵ are each independently selected from the group consisting of acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, pivaloyloxymethyl and t-butyryl.
 - 86. (Previously presented) A compound of formula (XII)

wherein R7 is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

 R^{10} is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are each independently selected from the group consisting of acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, pivaloyloxymethyl and t-butyryl;

with the proviso that R11, R14, and R15 are not all acetyl.

- 87. (Previously presented) The compound of claim 86, wherein R¹¹ is acetyl; and R¹⁴ and R¹⁵ are independently butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
- 88. (Previously presented) The compound of claim 86, wherein R¹¹ is butyryl, acetoxymethyl, propancyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl; and

R¹⁴ and R¹⁵ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

89. (Previously presented) The compound of claim 86, wherein $R^7 \text{ is -CH}_2\text{-}C_6\text{H}_6, \text{ naphthyl, -CH}_2\text{-}C_6\text{H}_4\text{OH, -CH}_2\text{-}C_6\text{H}_4\text{F, or -CH}_2\text{-}} \\ C_6\text{H}_4\text{OR}^{14}; \\ R^8 \text{ is -CH}_2\text{C}_6\text{H}_6, -\text{CH}_2\text{C}_6\text{H}_{11}, -\text{CH}_2\text{C}_6\text{H}_9, \text{ or -(CH}_2)}_3\text{NHC}(=\text{NH})\text{NH}_2; \text{ and } \\ R^9 \text{ is phenyl, indolyl, -C}_6\text{H}_4\text{OH, -C}_6\text{H}_4\text{NH}_2, -C}_6\text{H}_4\text{F, or -C}_6\text{H}_4\text{OR}^{16}. \\ \end{cases}$

90. (Previously presented) A compound of formula (XIII) or (XIV)

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₀H₄OR¹⁴;

 \mathbb{R}^{8} is H, alkyl, heteroalkyl, or aryl;

 R^{12} and R^{13} are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR 16 ; n is 0, 1, or 2; and

 R^{11} , R^{14} , and R^{16} are each independently acetyl, butyryl, acetoxymethyl, propancyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.